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EFFECTS OF CALCIUM AND POTASSIUM INJECTED INTO THE CEREBRAL VENTRICLES OF THE CAT

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In previous experiments (Feldberg & Sherwood, 1954*a*, *b*, 1955) injections of a number of drugs into the lateral cerebral ventricles of the unanaesthetized cat and the ensuing behavioural changes have been described. The present experiments are a continuation of this work and deal with the effects of intraventricular injections of calcium and potassium. These cations are known to exert powerful central depressant and excitatory effects respectively when given systemically or applied locally, but only a few experiments have so far been conducted in which the effects have been studied when these ions are introduced into the cerebral ventricles or into the great cisterna.

The effects on injection of calcium and potassium into the cerebral ventricles of cats were studied by Marinesco, Sager & Kreindler (1929). The salts were injected under light ether anaesthesia and the effects observed as the anaesthetic wore off. The injection of 1 mg calcium chloride caused, after a latency of about 1 hr, sleep which lasted for several hours. The injection of 1 mg potassium chloride resulted in clonic contractions which lasted for 20 min; afterwards the cats were unable to walk and were very excited, and about 2 hr after the injection they fell asleep for several hours, whilst respiration was accelerated and the body temperature raised.

Stern (1945) pointed out that an increase of the potassium:calcium ratio in the cerebrospinal fluid raised the tonus of the sympathetic nervous system and influenced the general state of excitation of the central nervous system. The intraventricular injection of a few milligrams of potassium into humans resulted in augmentation of cardiac activity, increased muscle tone and general excitability. Stern advocated the injection of a mixture of potassium dihydrogen and dipotassium hydrogen phosphates (2–7 ml. of a solution containing 6·5 mg potassium/ml.) for the treatment of shock. The mixture was 'introduced suboccipitally to the vegetative centres' in many war cases, and the result was satisfactory. In experiments on animals in a condition of shock,

the intraventricular injection of 0·1 ml./kg of such a solution led to a rise in arterial blood pressure, deepening of respiration and restoration of the excitability and reactivity of the animal.

METHODS

The method of injection into the cerebral ventricles of the cat through an indwelling cannula has been described previously (Feldberg & Sherwood, 1953). When 2 mg or more of either calcium or potassium chloride was injected intraventricularly, the calcium chloride was injected in a 0·9% solution and the potassium chloride in a 1·1% solution in distilled water. When smaller amounts than 2 mg were injected these solutions were diluted with saline solution and the injection volume was kept at 0·2 ml. In this way the salts were always injected in a solution which is practically isotonic with a 0·9% NaCl solution. In some experiments, in which adrenaline or noradrenaline was injected intraventricularly for comparison, the injection volume was also 0·2 ml.

RESULTS

Effect of calcium

The effect of an intraventricular injection of calcium chloride can be divided into two consecutive phases: the first is characterized by disturbances of balance, muscle power and posture, the second by anaesthesia. Both states are similar to those seen after an intraperitoneal injection of pentobarbitone.

The following is a general outline of the effects observed after an injection of 2 mg $CaCl_2$. Within about 20 sec the cat suddenly reared up, raised its head and shoulders and, staggering on its hind legs, barely avoided falling over backwards. The cat then slowly circled in the cage with an ataxic gait, hesitating between steps, missing its footing occasionally and then lying down, usually on its side. After a short time the cat rose again; each front leg was extended forward in turn while the hind legs remained flexed and performed joint, short, hopping movements; the cat lay down again and came to rest, at first keeping its head just off the floor, but later supporting chin or nose on a convenient object in the cage such as the rim of the food dish, or on the floor itself. It often braced itself against the side of the cage or against the food dish with the upper forelimb, when lying on its side. There were small alternating head movements from side to side, associated with nystagmoid movements of the eyes towards the side of deviation, and sometimes waves of fine oscillations of head and eyes. Otherwise, the cat seemed relaxed. At the onset of these signs the cat was alert, following the observer with its eyes, but within a few minutes the eyelids drooped and the nictitating membrane became visible to a variable extent; the general appearance resembled drowsiness or sleep. At this time the cat still reacted by opening the eyes a little for a short time when the observer approached the cage. Later the cat could no longer be roused and remained in a condition resembling full anaesthesia for 60–90 min. The cat then came out of this state gradually, full recovery not being achieved until several hours later; in fact, some lethargy was still present the following day.

The first signs of lightening of the state were reactions to noise, pin-prick and handling. Later the cat showed its increasing alertness by following events in the room with its eyes and holding its head raised in response to noise for lengthening periods, but as yet without rising from the recumbent position. At this and later stages weakness rather than drowsiness was the predominant feature, for when the cat resumed spontaneous locomotion, after 2–3 hr, it did so by reaching far out in front with one forepaw and then slowly following with the rest of the body without extending the hind legs, and it swayed from side to side. Still later it stretched its legs as a cat usually does after normal sleep, but showed signs of weakness by leaning against the side of the cage when progressing and by difficulties in initiating movements: before a few steps were taken there were a number of small hesitant movements in the direction of the subsequent steps. The cat also had difficulty in unflexing its hips, knees and heels and in maintaining a posture involving muscular effort, for instance, sitting upright. When the cat was tempted by a proffered hand to assume an upright position, it did so by grasping the side or roof of the cage with the forepaws, but the joints of both fore and hind limbs sagged, so that the cat, after hanging limply for a few seconds by one or both front paws, dropped to the floor.

Apart from this general behaviour the following more detailed observations and tests were carried out after the intraventricular injections of 2 mg $CaCl_2$.

Respiration. There was first an increase and then a decrease in the rate of respiration in the recumbent cat. The period of increased respiration was short, a maximum of 100–170/min was reached within 3–6 min. The rate of respiration then fell within the following half hour to values of 11–15/min. No significant rise in respiratory rate occurred during the following hour whilst the cat was lying immobile in the cage, and the rate did not change when unsuccessful attempts were made to rouse the animal. During the following hours the rate increased slowly even whilst the cat was lying inactive in the cage. When at this stage the cat was roused or resumed muscular activity, the rate rose naturally. At later periods, when the cat was responsive and active and rarely recumbent, no further counts of respiration were taken. The time course of the respiration rate following the intraventricular injection of 2 mg $CaCl_2$ is shown in Fig. 1. With repeated injections of $CaCl_2$, given at weekly intervals, the respiratory depression developed more quickly, was deeper and lasted longer.

Circulation. The injections caused bradycardia, which occurred at a time when the respiratory rate was still elevated. Minima of 70–90 beats/min were reached within the first 10–15 min; recovery began after a further 15–20 min. Normal values of 150 beats/min or more were again obtained about $1\frac{1}{2}$ hr after the injection.

The injection was also followed by an initial period of peripheral vaso-

dilatation, as shown by warmth of the nose, ears and pads of the paws. Vascular congestion of the ears was often pronounced and the colour, which was at first red or pink, assumed a bluish tinge at the time of maximal respiratory depression.

Oculo-motor signs. A few minutes after the injection the pupils became extremely narrow and later no light reflex, or only a slight one was obtained. There were periods of alternating small contractions and dilatations of the iris (hippus). The nictitating membrane became visible while the eyes were still half open, but never fully covered the eye. Later on the eyes were closed and

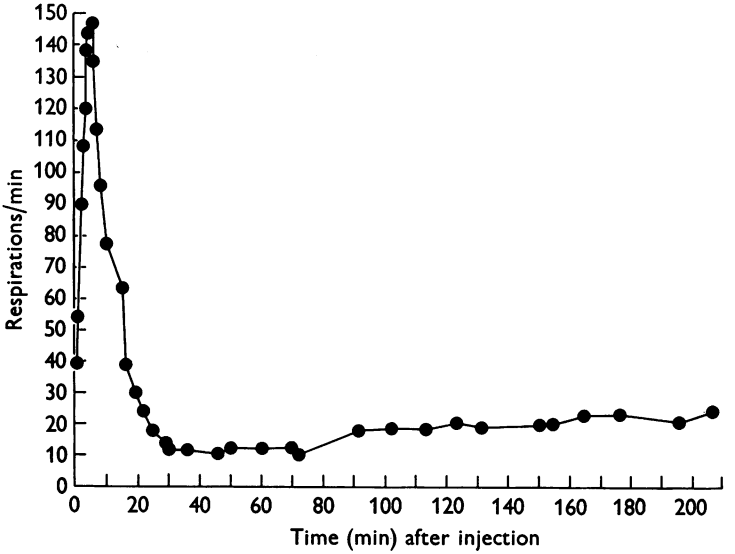

Fig. 1. Changes in the rate of respiration in an unanaesthetized cat after the injection of 2 mg $CaCl_2$ into the cerebral ventricle.

remained so for over an hour. Nystagmoid and oscillatory movements of the external eye muscles associated with head movements occurred early after the injection. Although spontaneous blink movements were rare or absent, the corneal reflex was not abolished, but it was less brisk.

Muscle tone, power and co-ordination. After the initial ataxia had become apparent the muscle tone decreased, and by the time the cat had lain down the muscles were completely relaxed so that on handling the cat conveyed the impression of a rag doll. The muscular tone returned gradually in the course of 1½–2 hr. Parallel to the loss of muscle tone there was a loss of muscle power, which was evident by the fact that the cat exerted only a weak traction when the paws were pinched at a time when the cat still responded with a sustained effort. When the cat was gently edged off a shelf from a height of about 4 ft. it resisted, bracing itself in the early stages against

the pushing hand, but later no such resistance was offered. Owing to ataxia and weakness its landing and righting became increasingly worse soon after the injection.

In order to test the ability to balance, the cat was placed on horizontal crossed bars at a height of about 4 ft. from the ground. Normally the cat has no difficulty in standing and moving about on them. Within 10 min after the injection the cat was unable to maintain its equilibrium on the bars, missed its foothold and made attempts to save itself by hanging on with the front paws until it dropped off and did not right itself during the fall. Later on the cat was too weak to make any efforts at all to save itself; as soon as it was placed on the bars it slid off and had to be caught. About 1½ hr after the injection the cat was still unable to balance on the bars but remained hanging with two or all four paws, made attempts to climb back and did not fall off. Three hours after the injection the cat could again stand and walk on the bars although it was still unsteady.

The placing reaction of all four paws was lost within 10–15 min after the injection; this condition was preceded by a period of inaccurate placing as indicated by overshooting of the movements. Partial return of the placing reaction occurred in the course of 1½ hr but the reaction was still indeterminate after 3 hr.

Reaction to deep pressure and pin-prick. In a normal cat deep pressure applied to a hind paw by pinching evokes immediate withdrawal. The cat tries to jerk the paw free, struggles and turns the head towards the affected limb. It miaows or shows signs of anger such as hissing, protruding its claws, or even biting. Within a few minutes after the injection these reactions became reduced and delayed and were absent after about 10 min; they returned gradually and were fully developed about 1½ hr later.

When the ear of a normal cat is pricked with a pin a light prick does not always produce a reaction but a heavier or sustained prick produces jerks and miaowing followed by vigorous shaking of the head and the performance of toilet, i.e., washing and licking itself. After the injection of $CaCl_2$ these 'displacement reactions' became first delayed and reduced, and later they were absent. At a time when the withdrawal reactions in response to deep pressure had returned, i.e. about 1½ hr after the injection, sustained and hard pricks produced no other reaction than the performance of 'toilet'.

Reaction to restraint and petting. A cat which normally did not accept restraint by being held by its shoulders between the observer's forearm and body for ½–1 min, remained quiet and did not try to wriggle free when the same test was applied a few minutes after the injection. Sometimes it made brief and feeble attempts to free itself. Similarly, a cat which normally reacted to petting by purring and pushing its head and sides against the stroking hand failed to react partially or completely a few minutes after injection. The response appeared again after about 1½ hr and was normal after about 3 hr.

Reaction to sudden noise. The reaction of a normal cat to a loud crash produced by dropping a large piece of aluminium sheet to the stone floor is as follows: the cat instantly winces and crouches down, remains fully immobile for several seconds and then shows little activity for some time, after which it often shows displacement reactions such as washing itself. After the injection this arousal became less marked and less enduring, the only effect being an attempt at raising the head, and finally even this no longer occurred. However, before this condition was reached, in the early stages of the $CaCl_2$ effects, the initial reaction was sometimes more violent than originally. The cat, instead of crouching down and becoming immobile, jerked violently and turned its gaze whilst eyes and pupils widened, before subsiding again.

When the intraventricular injection of 2 mg $CaCl_2$ was repeated within half an hour, in one cat the respiration stopped 20 min after the second injection and the heart beat became very slow. Artificial respiration, by massaging the thorax, had to be applied for 2–3 min before spontaneous respiration started again and the heart rate increased, but the respiratory rate remained below 20/min for over 1 hr.

Doses smaller than 2 mg of $CaCl_2$ were still effective. With 0·5 mg $CaCl_2$ a previously lively cat lay down and showed little spontaneous activity. It sometimes assumed the attitude of normal sleep preceded by narrowing of the pupils and drooping of the lids, which occurred from time to time whilst there was a general sagging of the head. The muscles were relaxed. The cat was easily roused but tended to doze off rather than to offer resistance to restraint. The injection of 0·5 mg $CaCl_2$ produced also an initial period of tachypnoea.

In some cats the effect of $CaCl_2$ was compared with that of an intraventricular injection of 200 μg of either adrenaline or noradrenaline, the effects of which have been described previously (Feldberg & Sherwood, 1954*a*). There were certain differences in the symptomatology: the respiratory changes and the initial stage of ataxia produced by $CaCl_2$ did not occur after adrenaline or noradrenaline, which sometimes produced retching and vomiting. The condition of the cat under adrenaline or noradrenaline differed from that under $CaCl_2$ by the greater depth and earlier onset of analgesia. Further, whereas under adrenaline muscular weakness, inco-ordination and ataxia were absent when the cat was in a condition to move, these signs were exhibited after $CaCl_2$ before the 'anaesthesia' developed and as it lightened again.

Effect of potassium

The effects of intraventricular injections of KCl were short-lasting. After 0·5 mg they lasted for less than 30 min, after 2–3 mg for about 40 min.

Immediately after the injection of 0·5 mg KCl the cat showed signs of agitation. It moved about in the cage with short darting movements which were well co-ordinated, and it constantly changed the direction of its gaze.

The appearance was one of heightened alertness. Attention was paid immediately to any slight sound or any movement of the observer. After a few minutes the cat often assumed a crouching position, the attitude of a prowling or lurking cat. A cat which previously used to toy with a paper ball thrown into the cage leapt at it and tore it up. However, when patted it would purr and it appeared friendly, showing that there was no basic change in its affect, as occurs for instance after small doses of bulbocapnine injected intraventricularly (Feldberg & Sherwood, 1955). Similarly, one cat which was of a timid nature and habitually cringed when approached showed no change in this habit after the injection.

Larger doses of KCl (2–3 mg) produced immediate retching. The cat then either crouched down and looked bewildered and frightened, with its whiskers pointing forward and its ears erect, or it was seized with tonic contractions at times in flexion and at times in torsion, preceded occasionally by circling in either direction. During the circling the cat was overcome by a spasm which caused it to fall over. The spasm consisted of arching of the back, extreme flexion of the limbs and backward rotation of the head over either shoulder. The cat maintained the abnormal attitude for a minute or two. The spasm was always tonic although there followed occasionally a few clonic twitches of the legs on either side but never bilaterally. During the spasm the muscles were hard and resisted passive extension, and the tone of the muscles remained raised after the spasm itself had passed. Later there were waves of fine tremor. When the cat was lifted by the scruff of its neck its hind legs extended abruptly instead of being drawn up as under normal conditions. One cat immediately after the injection of 3 mg KCl started to miaow hoarsely a few times, made a few circling movements and then scratched itself with the vigour of an uncontrollable paroxysm.

During the spells of spasm it was impossible to obtain accurate information of the cat's state of awareness. If awareness was abolished at all it could only have been for a very short period. Once the spasms had passed the cat's reaction to noises, patting or passive alteration of its posture was diminished, indicating that awareness at this stage was only partly impaired.

At the initial stages of the KCl effect there was sometimes a transient slight protrusion of the nictitating membranes and a dilatation of the vessels of the external ears. During the spasm the pupils were widely dilated.

DISCUSSION

The differences between the effects we obtained and those described by Marinesco *et al.* (1929) with intraventricular injections of $CaCl_2$ and KCl are probably explained by the difference in injection technique and by the complication of an immediately preceding ether anaesthesia in their experiments. They made the injections through a fresh puncture, and diffusion of the salts

across the area of the injury, and mechanical brain injury produced during the injection cannot be excluded by their method. Marinesco *et al.* state that $CaCl_2$ produces sleep after a latency of about 1 hr. In our experiments the condition produced by intraventricular injections of $CaCl_2$ resembles more a condition of anaesthesia and occurs early after the injection. Once the animal is motionless and no longer reacts to stimuli it is impossible to state anything beyond whether or not the cat can be roused, but the way the cat goes into this state after an injection of $CaCl_2$ shows a striking similarity to the early effects of pentobarbitone sodium.

In the previous experiments the effect of adrenaline and noradrenaline was compared with that of pentobarbitone sodium given intraperitoneally. It was now found that there is a closer similarity in the way the cat goes under after pentobarbitone sodium with the early stages of $CaCl_2$, since with adrenaline and noradrenaline, neither weakness, ataxia, nor inco-ordination were present. On the other hand, the initial transient tachypnoea and bradycardia obtained with $CaCl_2$ were also observed by Reitter (1957) in his recent experiments in which he produced in dogs, by intracisternal injections of adrenaline, anaesthesia deep enough for him to perform surgical operations.

The effects of intraventricular injections of $CaCl_2$, adrenaline and noradrenaline bring to mind the experiments of Hess (1944) in producing sleep on stimulation of the massa intermedia, in producing adynamia by stimulation of the lower and central parts of the hypothalamus and in producing atonia by stimulation of the lateral part of the hypothalamic regions, all of which can be looked upon as being part of the wall of the third ventricle. The effects of intraventricular injection suggest that sleep, anaesthesia and analgesia may be mediated through different although related neurological systems which are located close to the ventricular cavities.

The finding that the excitatory action of KCl, in comparison with that of tubocurarine, is shorter and slighter, may be due to the fact that the KCl diffuses rapidly, not only through the c.s.f. spaces but also through the adjacent tissues, or because tubocurarine forms a more stable attachment with the receptors of the affected cells than does KCl.

SUMMARY

1. The effects of calcium chloride and of potassium chloride injected into the cerebral ventricles were studied in unanaesthetized unrestrained cats.

2. The injection of calcium chloride (2 mg) produces initially a short period of tachypnoea, muscular weakness and ataxia followed by bradypnoea and an anaesthesia-like condition lasting for about $1\frac{1}{2}$ hr while the cat does not react to stimuli, including pain.

3. Apart from the initial tachypnoea no distinction can be made between this condition and the anaesthesia produced by intraperitoneal pentobarbitone

sodium, but the calcium chloride effect differs from that of intraventricular adrenaline or noradrenaline which is not associated with muscular weakness and inco-ordination but shows more pronounced analgesia before the full anaesthesia supervenes.

4. The injection of potassium chloride increases alertness and accelerates movements and in larger doses (2–3 mg) produces tonic seizures of short duration with or without a few localized clonic spasms; there follows a period of increased muscular tone. The observable effects last for 20–40 min.

5. The seizures produced by KCl differ from those produced by intraventricular tubocurarine by being mainly tonic and not fully generalized and in that awareness, if influenced at all, is only transiently and incompletely impaired.

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